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Prevention of Immunosuppression by Sunscreens in Humans Is Unrelated to Protection from Erythema and Dependent on Protection from Ultraviolet A in the Face of Constant Ultraviolet B Protection

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Sunscreens have been advocated as an important means of preventing skin cancer. Ultraviolet radiation induced immunosuppression is recognized as an important event in skin cancer development, yet the effectiveness of sunscreens in protecting the human immune system from ultraviolet radiation (i.e. ultraviolet radiation) is still unclear. The only currently accepted method of sunscreen rating is the sun protection factor system based on the prevention of erythema. We determined immune protection factors for six commercially available sunscreens using a nickel contact hypersensitivity model in humans. Both sun protection factor and immune protection factor testing was performed using the same solar simulated ultraviolet radiation source and dose–responses were used to determine endpoints both with and without sunscreens. We found that the immune protection factor did not correlate with the sun protection factor; however, immune protection factor was significantly correlated to the ultraviolet A protective capability of the sunscreens, indicating that sunscreen protection from ultraviolet A is important for the prevention of ultraviolet immunosuppression, when there is constant ultraviolet B protection. We recommend that sunscreens should be rated against their immune protective capability to provide a better indication of their ability to protect against skin cancer. ***Key words: sunscreening agents/ultraviolet rays. J Invest Dermatol 121:184–190, 2003***

Skin cancer is a common problem in those of European descent (Armstrong and Kricker, 1995). The main causative factor is ultraviolet radiation (UV), although the responsible wavelengths within this region are unclear. Animal experimentation has implicated UVB (wavelengths 290–320 nm) to be of particular importance, although UVA (wavelengths 320–400 nm) also plays a part in skin carcinogenesis (de Gruijl *et al*, 1993). Indeed UVA has been particularly implicated in both the opossum (Ley, 1997) and platyfish (Setlow *et al*, 1993) models of melanoma. Among its many effects on the skin, UV radiation (UVR) is immunosuppressive: it diminishes antigen-presenting cell function, induces immunosuppressive cytokine production, and abrogates both contact and delayed-type hypersensitivity reactions (Streilein *et al*, 1994; Beissert and Schwarz, 1999).

UV immunosuppression is an important event in skin carcinogenesis (Donawho and Kripke, 1991; Kripke, 1994). It is now well recognized that the chronically immunosuppressed organ transplant population is at increased risk of both melanoma (Jensen *et al*, 1999) and nonmelanoma skin cancers especially in sun-exposed sites (Lindelof *et al*, 2000). Exposure to sunlight and subsequent UV immunosuppression also enhances susceptibility to infectious agents (Jeevan *et al*, 1992; Norval *et al*, 1999).

Sunscreens have been used for over 30 y to protect from sunburn but despite this the incidence of skin cancer continues to rise. It is commonly believed that the use of sunscreens will prevent the development of skin cancer and, whereas there is evidence for this in animal models (Bestak and Halliday, 1996a), there is little evidence for this in humans (WHO, 2001). Two randomized trials have been performed that demonstrate that sunscreens can reduce the incidence of solar keratoses, which are believed to be skin cancer precursors (Thompson *et al*, 1993; Naylor *et al*, 1995). The only randomized control trial that has evaluated the efficacy of sunscreens against epithelial skin cancer showed that the daily use of sunscreens reduced the incidence of squamous but not basal cell carcinoma (Green *et al*, 1999). Trials of sunscreen protection from melanoma have produced inconclusive results (Wang *et al*, 2001) with some studies suggesting that melanoma is associated with sunscreen use (Garland *et al*, 1993). This could be due to UVA exposure being important in the development of melanoma (Ley, 1997) as sunscreens protect from UVB better than they protect from UVA.

Protection from erythema or sunburn by measurement of the sun protection factor (SPF) is currently the only internationally recognized endpoint for the evaluation of sunscreen effectiveness.

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Abbreviations: EI, erythema index; IPF, immune protection factor; MED, minimal erythema dose; MISD, minimal immunosuppressive dose; MPD, minimal persistent pigment darkening dose; SPF, sun protection factor; ssUVR, solar simulated ultraviolet radiation; UVA-PF, UVA protection factor.

Erythema is primarily caused by UVB (Farr and Diffey, 1985) and, therefore, sunscreens that protect well from UVB but not UVA effectively prevent sunburn. Studies that have compared the ability of sunscreens to protect against erythema and immunosuppression have so far yielded conflicting results (reviewed in Ullrich *et al*, 1999) (Roberts and Beasley, 1995; Bestak and Halliday, 1996a; Moyal *et al*, 1997; Serre *et al*, 1997; Walker and Young, 1997; Fourtanier *et al*, 2000). Most studies only examined one or two sunscreens. Few studies can be compared with each other as they have not used the same UV spectral source and method of sunscreen application to test the various products, both of which can alter sunscreen performance (Stenberg and Larko, 1985; Roberts *et al*, 1996). Most importantly, the level of protection from immunosuppression in humans, which can only be determined by dose–responses has only been evaluated in a single study of two sunscreens (Damian *et al*, 1999a). It is important that the level of both immune and erythema protection based on UV dose–responses are measured using identical experimental conditions to resolve the issue of whether the SPF predicts protection of the immune system in humans (Young and Walker, 1999) and to evaluate the effect of spectral absorbance by the sunscreen on immune protection.

This study therefore compared the immune and sunburn protection of a range of commercially available sunscreens using the same UV source and sunscreen application density. We used a previously described *in-vivo* human nickel contact hypersensitivity recall model (Damian *et al*, 1997) of UV-induced immunosuppression to obtain UV dose–responses both with and without sunscreens and to determine sunscreen immune protection factors (IPF). In addition, we examined the relationship between sunscreen UVA protective capability and immune protection.

MATERIALS AND METHODS

Subjects Ninety-nine nickel allergic volunteers aged 18–71 y (mean 35.5 y) were recruited by advertisement from the general population for IPF studies. None of the volunteers were on immunosuppressive or anti-inflammatory medications and they did not have any sun exposure to their backs for at least 4 wk prior to the study. A separate group of 64 non-nickel allergic volunteers was used for sunscreen SPF testing and 34 for the sunscreen UVA protection factor (UVA-PF) studies. Approval was obtained from both the Central Sydney Area Health Service and University of Sydney Ethics Committees in accordance with the Helsinki Principles. All volunteers gave their informed consent for the studies.

UV source The same UV source was used for both SPF and IPF determinations. This was an Oriel 1000 W ozone-free xenon arc lamp (Oriel, Stratford, Connecticut) filtered with two 280–400 nm dichroic mirrors (Oriel) to reduce the visible and infrared output and an atmospheric attenuation filter (Oriel, serial number 81017) to approximate closely solar UV (**Fig 1**). Irradiance was monitored with a scanning spectrophotometer (Optronics, Orlando, Florida) and UV output monitored daily with an IL1350 broadband radiometer using SED 038 (UVA) and SED 240 (UVB) detectors (International Light, Newburyport MA, USA) calibrated against the source with the spectrophotometer. The integrated irradiance at the skin surface was 3.4 mW per cm^2 UVB, 6.9 mW per cm^2 UVA II, and 19.2 mW per cm^2 UVA I.

For the UVA-PF studies, an Oriel 1000 W xenon arc was used, however, instead of the atmospheric attenuation filter, a UVC and UVB blocking filter (WG320, Oriel) was used to produce a UVA only spectrum with a cut-off at 320 nm (**Fig 1**).

Sunscreens The six commercially available sunscreens were purchased in Australia in the year 2000 and were all labeled as broad spectrum. Their ingredients are shown in **Table I**.

***In-vivo* SPF determination** The Australian guidelines for SPF testing were followed (Standards Australia, 1998), with 10 volunteers tested per sunscreen. Sunscreen was applied at a density of 2 mg per cm^2 and allowed to dry for at least 15 min prior to irradiation. Test sites on both sunscreen-protected and unprotected lower-mid back skin received a single exposure to a range of doses of UV. The minimal erythema dose (MED) was determined as the lowest UV dose that resulted in just perceptible erythema with clearly defined borders 24 h later. The SPF was calculated as the ratio of the MED of sunscreen-protected skin with that of unprotected skin in each volunteer.

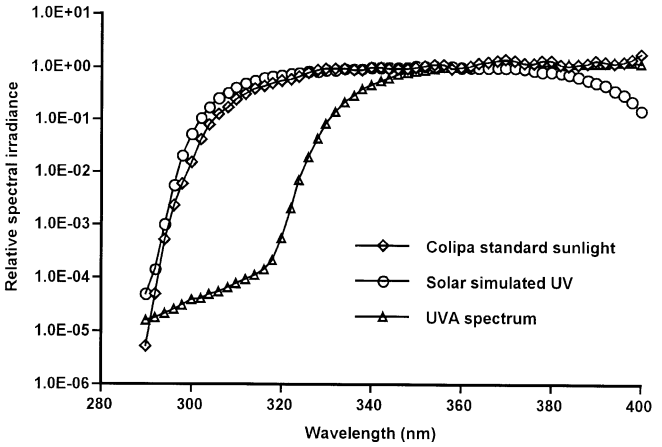

Figure 1. Spectral output of UV source. The Oriel 1000 W solar simulator and filters used in this study provided a good approximation to natural sunlight as defined by COLIPA (1994) in the UV range. The relative spectral outputs are very similar in the UVB region, whereas approximation in the UVA region does not differ substantially until the longer UVA wavelengths are reached. This spectra lies within the Australian standard range (Standards Australia, 1998) for ssUV spectra. The UVA spectrum used for the UVA-PF studies is also shown.

Table I. Sunscreen ingredients

	Sunscreen[b]					
Ingredient[a]	A	B	C	D	E	F
Titanium dioxide	8.3		4		2	3
Zinc oxide		7.5				
Oxybenzone	2.5					
Octylmethoxycinnamate	9	8	6	8.5	7.5	
4-tert butyl-4-methoxybenzoylmethane				2	2	4.2
4-methylbenzylidene camphor		2.5			5	
Octocrylene						10.5

[a]The ingredients (%w/w) for each test sunscreen as found on the sunscreen bottles.
[b]All sunscreens were purchased commercially and were labeled as broad spectrum, containing both UVB and UVA protective ingredients.

***In-vivo* UVA-PF determination** The same sunscreen application method and UV irradiation protocol as for SPF testing was used for the UVA-PF determinations except that a UVA-only spectrum was used. Each sunscreen was tested on 10 volunteers. The results were read 2 h after irradiation and the minimal persistent pigment darkening dose (MPD) was defined as the lowest UV dose that produced barely perceptible tanning with clearly defined borders. The UVA-PF was calculated as the ratio of the MPD of sunscreen-protected to unprotected skin as described by Chardon *et al* (1997).

***In-vitro* sunscreen absorbance determination** The spectral absorbance profiles of each sunscreen were obtained, using a Labsphere UV-1000 SPF analyzer (North Sutton, New Hampshire) with sunscreen applied at 2 mg per cm^2 on to a quartz plate substrate profiled with the topography of human skin derived from casts of human test back skin. Two different methods of rating UVA protection were calculated from the absorbance spectra. The Diffey critical wavelength is that wavelength below and including which 90% of the total UV is absorbed by a sunscreen (Diffey, 1994). Higher critical wavelengths therefore indicate better UVA protection. The Boots UVA ratio is the ratio of the total absorption by a sunscreen in the UVA region compared with that in the UVB region.

***In-vivo* IPF determination** *In-vivo* IPF were determined as we have previously described in detail (Damian and Halliday, 2002). Two 6 × 6 cm sites were demarcated on each nickel allergic volunteer's mid-lower back (**Fig 2**). One site was randomly allocated to have sunscreen applied using the same density and method as for SPF and UVA-PF testing. The contralateral area contained four segments that received four different doses of solar-simulated UVR (ssUVR; unprotected UVR), one unirradiated control that did not receive sunscreen (positive control), and one unirradiated control treated with sunscreen (positive sunscreen control). The sunscreen-protected site was divided into six segments each of which received a different dose of ssUVR (sunscreen UVR). These UV doses and sunscreen treatments were repeated daily on 4 consecutive days, with the same dose being delivered to a particular site each day. All subjects protected with a particular sunscreen received the same set of UVR doses, regardless of skin type or individual MED. Sunscreen was applied each day and washed off with soap and water following irradiation.

Immediately following the final irradiation, 9 mm Finn chambers (Epitest, Tuusula, Finland) containing nickel sulfate in a petrolatum base (Trolab Hermal, Reinbeck, Germany) were applied to each of the sites and removed after 48 h. Nickel concentrations were individualized by prior patch testing of each volunteer. Doses that produced confluent erythema without vesiculation were used in each volunteer. Twenty-four hours later, the nickel induced erythema was read using a reflectance spectrometer (Diastron, Hampshire, UK). The erythema index (EI) of each nickel test site was calculated as the difference of the average of four readings taken at each test site and the average of four readings taken from adjacent skin.

Data analysis Immunosuppression was calculated at each test site by subtracting the nickel-induced EI at that test site from the EI of the unirradiated positive control to determine immunosuppression in erythema units. The results of 15 volunteers for each sunscreen were pooled at each UV dose and used to determine UV dose–response curves with and without sunscreen protection. The level of immunosuppression for both sunscreen protected and unprotected sites was plotted against UV dose and linear regression was performed to produce a line of best fit. Paired two-tailed Student's t tests were used to compare nickel-induced erythema at each test site with the unirradiated positive control site to determine whether the immunosuppression reached statistical significance: results were considered significant if $p < 0.05$.

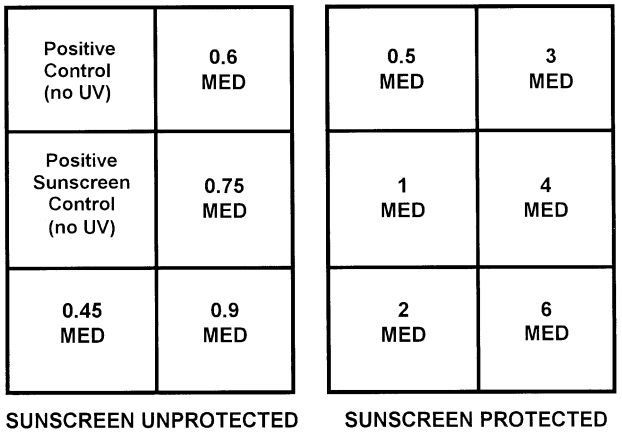

Figure 2. Example of UV irradiation doses for IPF testing. Schematic diagram of a volunteer's lower mid-back showing an example of UVR irradiation doses given each day for the four irradiation days. All subjects within an experiment received the same doses of UVR regardless of skin type. One 6 × 6 cm area was randomly allocated as the sunscreen-unprotected side with the contralateral area allocated as the sunscreen-protected side. Each area was divided into six different segments. UVR doses were chosen according to the SPF of each sunscreen. This example gives the UVR irradiation doses for an SPF 6 sunscreen. The average MED is 243 J per m^2. Immediately following the final irradiation, nickel patches were applied to each segment. The positive control segment received neither sunscreen nor UV, the positive sunscreen control received sunscreen in the absence of UV.

Reduction in the nickel-induced EI of irradiated sites equal to 30% of the mean EI of the unirradiated positive control for that experimental group was considered to be the lowest level of immunosuppression that could be reliably and reproducibly detected using this method. The dose of UV that caused this level of immunosuppression was therefore considered to be the minimal immunosuppressive dose (MISD), which is analogous to the MED in SPF testing. This dose of UVR, the MISD, was calculated from the linear regression analysis of the data. The IPF for each sunscreen was calculated as the ratio of the MISD of sunscreen protected to sunscreen-unprotected skin.

Statistical analysis of correlations between SPF, IPF, and other parameters were performed using a Fisher's R to Z-test (Statview statistical software, Abacus Concepts, Berkeley, CA, USA) and results were considered significant if $p < 0.05$.

RESULTS

Unirradiated nickel control reactions There were no significant differences between the unirradiated positive controls and the unirradiated positive sunscreen controls for any sunscreen (paired Student's t test). Thus none of the sunscreens on their own affected the nickel reaction.

Calculation of MISD and IPF For each sunscreen, IPF were calculated from the pooled results of 15 volunteers, as shown in **Fig 3** for sunscreen D. Fifteen volunteers were irradiated with the doses of UV shown on separate segments of sunscreen-protected or unprotected skin. Each volunteer received the same UV dose for each of 4 consecutive days. The nickel-induced contact sensitivity response was quantitated with the reflectance spectrometer and immunosuppression was calculated at each UV dose in each volunteer as the difference between the unirradiated positive control and the test site. The mean immunosuppression of the 15 volunteers at each UV dose was plotted against UV dose and a line of best fit calculated by linear regression. To be consistent between experiments with each sunscreen, 30% of the positive control for each experiment was used as the cut-off because the immunosuppression at this point was the minimum level that was statistically significant in all experiments ($p < 0.05$, paired Student's t test). For sunscreen D shown in the example, the mean of the positive control was 73.1 erythema units and therefore 21.9 (30% of 73.1) erythema units was the cut-off. The

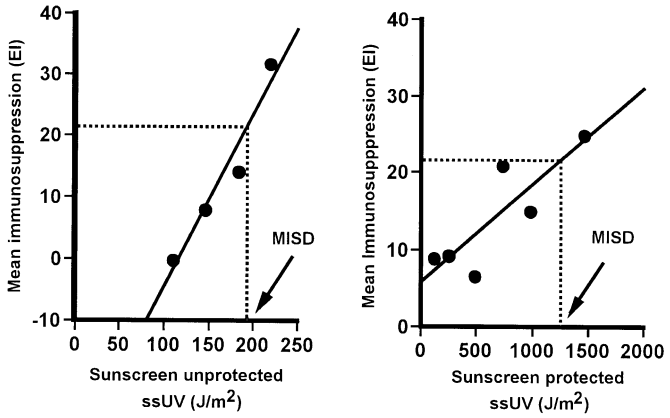

Figure 3. Calculation of MISD and IPF for sunscreen D. UV dose–response curves both with and without sunscreen protection were determined by linear regression analysis using data pooled from 15 subjects. The average of the positive controls was 73.1 erythema units. A cut-off of 30% was chosen as the lowest level of significant immunosuppression that could be reliably determined and thus 30% of 73.1 = 21.9 EI. The MISD of both sunscreen protected and sunscreen unprotected sites was thus the UV dose which caused an immunosuppression of 21.9 EI. These were determined from the linear regression equation and the IPF then was the ratio of these MISD.

UV dose that caused this level of immunosuppression (MISD) was calculated from the linear regression analysis and the IPF was the ratio of MISD (sunscreen protected) to MISD (unprotected). The IPF was calculated in the same way for each sunscreen and the results are shown in **Table II**.

Broad-spectrum ranking of sunscreens A summary of the IPF, SPF, and UVA-PF of all sunscreens is shown in **Table II**. SPF and IPF were measured with the same UV source and other conditions. The SPF supplied by the manufacturer was not used in these studies. The sunscreens were ranked according to their UVA-PF. As there is currently no internationally accepted standard of assessing UVA protection, we used three methods that have been proposed as standards, namely the Diffey critical wavelength method, the Boots UVA ratio, and the UVA-PF using persistent pigment darkening. A similar order to the Diffey critical wavelength was obtained with the Boots UVA ratio, except that the ranking of the two sunscreens with the least UVA protection, sunscreens A and B, were reversed; however, using the UVA-PF, the order of the sunscreens did not correlate as well with either the Diffey or Boots methods.

There is no correlation between sunscreen IPF and SPF The *in-vivo* SPF for each sunscreen was not predictive of the sunscreen's IPF obtained using the nickel contact hypersensitivity model as there was not a significant correlation between these protection factors (**Fig 4**). Thus immune protection was independent of erythemal protection. The range of SPF was between 6 and 20, whereas the range of IPF was between 2 and 21 (**Table II**). The sunscreen with the highest SPF (E) did not have the highest IPF (F), whereas the sunscreen with the lowest SPF (D) did not have the lowest IPF (A). Both sunscreens A and C have approximately the same SPF (11 and 10, respectively), whereas sunscreen C has an IPF approximately four times that of sunscreen A. Sunscreen A has approximately twice the SPF as sunscreen D (11 *vs* 6), yet sunscreen D has three times the IPF of sunscreen A. Conversely, sunscreens B and C have similar IPF (10 *vs* 9) but they have different SPF (15 *vs* 10).

Sunscreen IPF but not SPF correlates with sunscreen UVA protection A significant positive correlation was observed between IPF and the Diffey critical wavelength ($p < 0.05$) (**Fig 5**). Similarly, there was also a significant positive correlation between IPF and the Boots UVA ratio ($p < 0.05$) (**Fig 5**). Both of these parameters measure the breadth of a sunscreen's protection and thus show that the spectral broadness of a sunscreen is an important factor for immune protective capability. In addition, there was a significant positive correlation between IPF and UVA-PF ($p < 0.001$) this indicates the importance of protection from UVA in determining sunscreen immune protection (**Fig 6**). Conversely, linear regression analysis showed that there were no significant correlations between SPF and either the Diffey critical wavelength, Boots UVA ratio, or UVA-PF. These data indicate that UVA protection is more important for immune protection than it is for erythemal protection.

DISCUSSION

This is a study to determine the limit of sunscreen protection to the immune system based on dose–responses in humans for sufficient numbers of sunscreens to study the issues that are important for a sunscreen to protect the immune system. A single previous study (Damian *et al*, 1999a) determined IPF for two sunscreens,

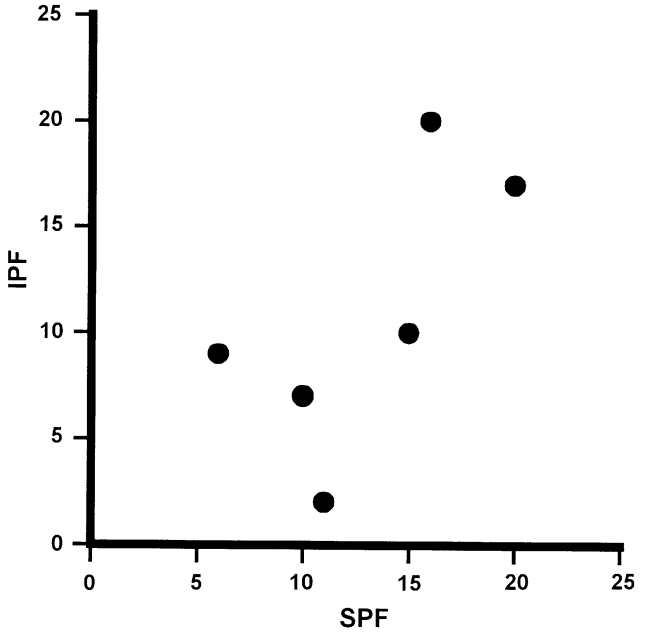

Figure 4. IPF does not correlate with SPF. The SPF and IPF of the six test sunscreens were plotted against each other. Each point represents a different sunscreen. There was no significant correlation between SPF and IPF (Fisher's R to Z-test; $r^2 = 0.53$, $p > 0.05$) indicating that the immune protection of a sunscreen cannot be predicted from its SPF.

Table II. *In-vivo* sunscreen SPF and IPF results compared with Diffey critical wavelength, Boots UVA ratio and UVA-PF

			UVA protection factors[c]		
Sunscreen	SPF[a]	IPF[b]	Diffey critical wavelength[d]	Boots UVA ratio[e]	UVA-PF[f]
A	11	2	353	0.36	1.6
B	15	10	368	0.33	2.3
C	10	9	373	0.47	2.8
D	6	7	375	0.52	1.9
E	20	17	378	0.61	4.7
F	16	21	383	0.85	4.5

[a]SPF and IPF were measured using the same solar simulator system and conditions. SPF was measured following the Australian Standard with the average results of 10 volunteers per sunscreen.
[b]IPF was determined using groups of 15 volunteers for each sunscreen as shown in the example in **Fig 3**.
[c]Both the Diffey critical wavelength and Boots UVA ratio were determined from the *in-vitro* absorption spectra. The UVA-PF was determined *in-vivo* by the persistent pigment darkening method using a UVA-only spectrum for irradiation of the volunteers.
[d]The Diffey method calculates the wavelength below which 90% of a sunscreen's absorbance occurs.
[e]The Boots UVA ratio is the ratio of a sunscreen's total UVA absorbance compared with its total UVB absorbance.
[f]The UVA-PF indicates the level of UVA protective capability of a sunscreen and was determined by the persistent pigment darkening method.

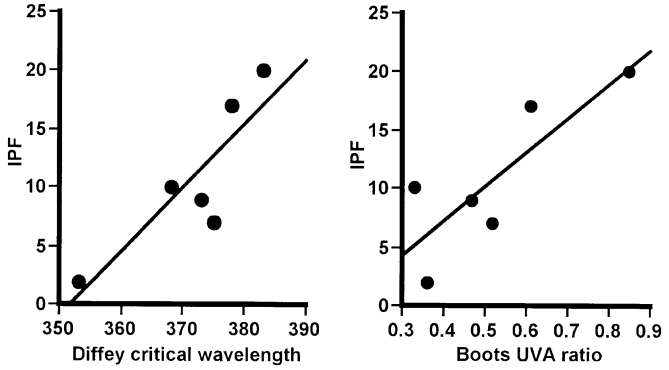

Figure 5. IPF correlates with Diffey critical wavelength and Boots UVA ratio. IPF was plotted against Diffey critical wavelength or Boots UVA ratio. Each point represents a different sunscreen. There were significant correlations between IPF and both the Diffey critical wavelength (Fisher's R to Z-test; $r^2 = 0.71$, $p < 0.05$) and the Boots UVA ratio (Fisher's R to Z-test; $r^2 = 0.72$, $p < 0.05$), which indicate that the breadth of a sunscreen's protective capability is important in determining immune protection.

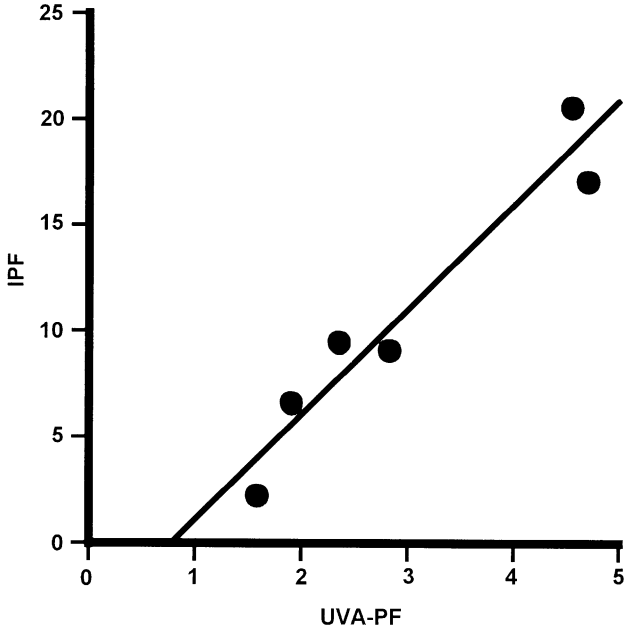

Figure 6. IPF correlates with UVA-PF. There was a significant correlation when IPF was compared to the UVA-PF (Fisher's R to Z-test; $r^2 = 0.93$; $p < 0.001$) indicating that UVA protection of a sunscreen is an important determinant of immune protection.

demonstrating that the methodology was appropriate; however, insufficient numbers of sunscreens were analyzed to dissect the sunscreen properties that influence immune protection. These properties have important implications for sunscreen product design and protection from skin cancer. We have demonstrated that a sunscreen provides better protection against immune suppression than erythema if it possesses exceptional UVA protection. This provides a scientific rationale for designing sunscreens that protect from UVA as they may provide better protection from skin cancer.

There did not appear to be any association between a particular sunscreen ingredient and IPF. de Fine Olivarius *et al* (1999) demonstrated that a sunscreen that contained a chemical UV blocker was more effective at preventing the isomerization of *trans*-urocanic acid than a sunscreen containing a physical blocker (titanium dioxide) with the same SPF. Whereas this is an important immunosuppressive mechanism, in our study the worst performing sunscreen (sunscreen A) and the best performing sunscreen (sunscreen F) both contained titanium dioxide and chemical agents. Sunscreen F, however, which was the only sunscreen to have an IPF greater than its SPF, was the only one to contain octocrylene. Interestingly, sunscreen F was also the only sunscreen not to contain octylmethoxycinnamate. Sunscreen A, which performed worst with regard to immune protection was also the only sunscreen to contain oxybenzone. It has been suggested previously that the addition of anti-oxidants such as vitamin E may provide additional UV protection to the immune system (Yuen and Halliday, 1997) and it is therefore possible that anti-oxidants in the sunscreens contributed to the IPF. Therefore an examination of whether immune protection is related to particular sunscreen ingredients would require a more systematic study of this issue using specifically formulated sunscreens.

We did not find any correlation between a sunscreen's ability to protect against immunosuppression and erythema. This has been a highly controversial area, which due to a lack of dose–response data required to establish limits of protection, has not been previously resolved. Most of the original studies in mice observed that sunscreens either failed to protect (Reeve *et al*, 1991) or only partially protected (Wolf *et al*, 1993) against UV-induced immunosuppression compared with erythema. The UV sources in these early studies were often unfiltered FS-type lamps that contained contaminating UVC. Using appropriate ssUV sources and methods based on those used for SPF testing, Roberts and Beasley (1995) found that commercial sunscreens were able to protect the immune system at a level exceeding the labeled SPF in mice. It is important to note that the labeled SPF would have been derived from human studies (which were not confirmed using the same UV source), yet mice were used to obtain immunosuppression data. *In-vitro* dose–response studies performed by Peguet-Navarro *et al* (2000) on human skin explants using the mixed epidermal cell reaction found that the IPF for four sunscreens ranked similarly to their SPF. This study, however, utilized only a UVB source at 312 nm ignoring the possible effects of UVA on IPF.

Whereas this is the first study to determine immune protection limits based on dose—response curves in a sufficient number of sunscreens to address the factors involved, our findings are in agreement with conclusions based on findings from single UV dose studies (Bestak *et al*, 1995; Moyal *et al*, 1997). These studies also found that the SPF cannot be considered a satisfactory guide to sunscreen immune protective capacity. Considering that the action spectra for erythema and immune suppression are probably different, this would not be an unexpected result. UVB is primarily responsible for erythema (Farr and Diffey, 1985), whereas the action spectrum for immunosuppression in humans is not known.

The role of the UVA waveband in UV-induced immunosuppression is controversial. Some studies find that UVA is immunosuppressive, whereas others find that it is immunoprotective. Bestak and Halliday (1996b) demonstrated that UVA radiation was capable of causing a significant reduction in the number of Langerhans cells from the epidermis of mice and of inducing immunosuppression. Moyal *et al* (1997) examined the effects of UVA on the delayed type hypersensitivity response in humans. Both UVB together with UVA and UVA alone caused immunosuppression both locally and systemically. Using the nickel contact hypersensitivity model in humans used in this study, we have previously shown that the time course for UVA-induced suppression differed from the time course for UVB immunosuppression (Damian *et al*, 1999b) and that additional UVA can augment ssUV-induced immunosuppression (Kuchel *et al*, 2002). Dumay *et al* (2001) performed *in-vitro* studies using epidermal cell suspensions specifically investigating the importance of long-wavelength UVA in immunosuppression. They found that UVA I

exposure resulted in both Langerhans cell depletion and downregulation of antigen presenting cell activity.

In contrast, earlier studies into the action spectrum for UV immunosuppression by De Fabo and Noonan (1983) and Elmets *et al* (1985) did not demonstrate the importance of UVA in immunosuppression. These studies, however, used UV sources that were contaminated with UVC and did not actually investigate wavelengths above 320 nm. Studies by Reeve *et al* (1998) showed that UVA was able to protect against UVB-induced immunosuppression in hairless mice. Skov *et al* (2000) also demonstrated that UVA I was able to partially protect against UVB-induced reduction in immunization rates in humans to epicutaneously applied antigens. These studies, showing an immunoprotective effect of UVA, used very high UVA doses. Recently our group has shown that, whereas low-dose UVA causes immunosuppression, high doses are immunoprotective, at least in some mouse strains (Byrne *et al*, 2002).

The sunscreens used in this study all protected well in the UVB region but had different UVA protective abilities as indicated by their different UVA protection indices. As both UVB and UVA are immunosuppressive, it is likely that all sunscreens used in this study provided adequate protection from the UVB doses used in this study, so that the ability to protect in the UVA waveband was the limiting factor in preventing immune suppression from ssUV.

Our findings that IPF correlates with both the Diffey critical wavelength and Boots UVA ratio clearly demonstrated the importance of the breadth of sunscreen UVB and UVA protection in order to protect the immune system. This is in agreement with several previous sunscreen studies that showed that broad-spectrum sunscreens protected better than UVB-only sunscreens against single doses of UV. The first study to suggest this was in mice, where a broad-spectrum sunscreen provided greater protection from a single dose of UV (Bestak *et al*, 1995). This was later confirmed in humans (Damian *et al*, 1997) again using a single dose of UV.

Our results showing the important relationship between immune and UVA protection as determined by UVA-induced persistent pigment darkening extends earlier studies by Fourtanier *et al* (2000), who compared two sunscreens with the same SPF but different UVA-PF to show that the product with the higher UVA-PF more efficiently prevented suppression of contact hypersensitivity in mice. These results were confirmed in a later study that showed that the suppression of delayed type hypersensitivity by full spectrum UVA, UVA I, or ssUV could be prevented by a sunscreen with a high UVA-PF better than one with lower UVA protection (Moyal and Fourtanier, 2001). A more recent study (Nghiem *et al*, 2001) showed that UVA was as effective as ssUVR in suppressing the elicitation of an established immune response to *Candida albicans* in mice. A sunscreen containing both UVB and UVA filters was able to completely prevent this effect, whereas a UVB-only filter provided no protection. These studies, however, did not enable the detailed analysis that is only possible with dose–responses.

There is now a large body of evidence confirming the importance of UV-induced immunosuppression in the development of skin cancer. We have demonstrated that for a sunscreen to provide good protection against immunosuppression (i.e., have a high IPF) it must provide good UVA protection. The importance of UVA in the etiology of melanoma has been suggested by Wang *et al* (2001) yet there is controversy as to whether sunscreen use is a risk factor for the development of melanoma. It is possible that prolonged sun exposure enabled by the use of sunscreens with poor UVA protection results in high levels of immunosuppression, which then contributes to skin cancer development.

In view of the results of this study, we would recommend that sunscreens should be labeled not only with an SPF, but also an IPF, to give a more accurate assessment of protection from the damaging effects of UV. Even though we have shown that the IPF increases as UVA-PF increases, other factors such as sunscreen ingredients and other unknown factors may influence the preventive effects from immunosuppression. Hence at this stage it would be prudent to assess IPF as well as UVA protection for sunscreens. A standard protocol for IPF determinations would need to be agreed upon, which had biologic endpoints that were relevant to human carcinogenesis. This may aid the development of sunscreens with improved protection from skin cancer.

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